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An Analytical Method for the Measurement of Nonviable Bioaerosols

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ABSTRACT

Exposures from indoor environments are a major issue for evaluating total long-term personal exposures to the fine fraction (<2.5 μm in aerodynamic diameter) of particulate matter (PM). It is widely accepted in the indoor air quality (IAQ) research community that biocontamination is one of the important indoor air pollutants. Major indoor air biocontaminants include mold, bacteria, dust mites, and other antigens. Once the biocontaminants or their metabolites become airborne, IAQ could be significantly deteriorated. The airborne biocontaminants or their metabolites can induce irritational, allergic, infectious, and chemical responses in exposed individuals.

Biocontaminants, such as some mold spores or pollen grains, because of their size and mass, settle rapidly within the indoor environment. Over time they may become nonviable and fragmented by the process of desiccation. Desiccated nonviable fragments of organisms are common and can be toxic or allergenic, depending upon the specific organism or organism component. Once these smaller and lighter fragments of biological PM become suspended in air, they have a greater tendency to stay suspended. Although some bioaerosols have been identified, few have been quantitatively studied for their prevalence within the total indoor PM with time, or for their affinity to penetrate indoors.

This paper describes a preliminary research effort to develop a methodology for the measurement of nonviable biologically based PM, analyzing for mold and ragweed antigens and endotoxins. The research objectives include the development of a set of analytical methods and the comparison of impactor media and sample size, and the quantification of the relationship between outdoor and indoor levels of bioaerosols. Indoor and outdoor air samples were passed through an Andersen nonviable cascade impactor in which particles from 0.2 to 9.0 μm were collected and analyzed. The presence of mold, ragweed, and endotoxin was found in all eight size ranges. The presence of respirable particles of mold and pollen found in the fine particle size range from 0.2 to 5.25 μm is evidence of fragmentation of larger source particles that are known allergens.

IMPLICATIONS

Major indoor air biocontaminants include mold, bacteria, dust mites, and other antigens. This project was a preliminary research effort to examine biologically based PM, analyzing for mold, mycotoxins, dust mites, and ragweed antigens and endotoxins. The objectives included quantifying the relationship between outdoor and indoor levels of biologically based component PM, and determining the size fraction of indoor/ambient PM that is biological. The findings can be important in explaining the exposure mechanism for biocontaminants and the size fraction that must be addressed by building owners and managers, as well as guidance for policy-makers.

INTRODUCTION

Studying the relationship between indoor and outdoor bioaerosols is important and necessary to the understanding of transport and deposition of biologically based contaminants in the indoor environment, where exposure is greatest. We spend in excess of 90% of our time indoors[1] and, consequently, receive a significant fraction of our exposure within that environment. Exposures to contaminants within the indoor environments are a major issue for evaluating total long-term personal exposures to the fine fraction (<2.5 μm in aerodynamic diameter) of particulate matter (PM). PM may originate from an ambient

source and penetrate into the indoor environment, or it may originate from an indoor source. One approach to distinguishing indoor and outdoor sources is to evaluate the differences in the characteristics of ambient and indoor PM.

It is widely accepted in the indoor air quality (IAQ) research community that biocontaminants are one of the important indoor air pollutants. Airborne biocontaminants or their metabolites make up the portion of PM that is biological PM (BioPM)[2] and can induce allergic, toxic, and infectious responses in exposed individuals. Symptoms of exposed individuals include coughing, wheezing, runny nose, irritated eyes or throat, skin rash, diarrhea, aggravation of asthma, headache, and fatigue. Immunological reactions can include asthma, allergic rhinitis, and hypersensitivity pneumonitis. In addition, the exposure of children to *Stachybotrys chartarum* is under investigation for an association with pediatric pulmonary hemorrhage.[3,4]

PM of biological origin has been shown by Salvaggio and Aukrust[5] to be made up of fungi, bacteria, plant pollen, and spore material, all of which have been linked to allergic symptoms. Although pollen is widely studied as an aeroallergen, little is known about ambient concentrations of fungal spores. Salvaggio and Aukrust[5] found *Cladosporium* spores in ambient air, at a concentration 1000 times the concentration of pollen grains. Aerometric sampling devices have collected spores from 20,000–40,000 species of fungi. Of these, four major groups have been identified as being potential allergens: *Phycomycetes*, *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes*.[5]

Bacteria and fungi are important components of outdoor, or atmospheric, aerosols, in addition to being important components of indoor aerosols. The size range of airborne bacteria is from 0.5 to 2.0 μm (*Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp., and *Arthrobacter* spp.), while many mold spores are significantly larger—2.5–3.0 μm for *Aspergillus fumigatus*, 3.5–5.0 μm for Aspergillus niger, 3.0–4.5 μm for *Penicillium brevicompactum*, 7–17 × 5–8 μm for *Cladosporium macrocarpum*, 15.0–25 μm for *Epiccocum nigrum*, and 2.8–3.2 μm for *Trichoderma harizanum*.[6] In addition, many species of pollen have been found to exceed 10 μm in size, with significant seasonal variation in variety. However, desiccated nonviable fragments of organisms are also common. These fragments can remain toxic or allergenic, depending upon the specific organism or organism component. The organism components can cause allergic, asthma-like reactions or pulmonary disease upon exposure. Although these bioaerosols have been identified, they have not been quantitatively studied for their prevalence in PM with time or their affinity to penetrate indoors. The range in size, being 0.1–10 μm for these fragmented biologically based particles or BioPM, readily allows resuspension into indoor air with occupant activity, as well as an affinity to stay airborne, posing a significant chance of exposure to occupants. The quantitative identification of BioPM is therefore the focus of this research effort.

Little information is available that quantifies the relationship between indoor and outdoor levels of bioaerosols. Although it is well established that outdoor levels influence indoor levels, the mechanisms are not understood. Outdoor levels of bioaerosols are affected by a number of factors, including seasonal variation, environmental or climatic influences, and activity in the area. As in the study of PM, penetration transfer mechanisms for bioaerosols from outdoors include infiltration, ventilation flows, and mechanical transport on apparel. Over some period of time, indoor levels are thought to be dependent on outdoor levels when indoor sources are minimized. Bioaerosols have been identified outdoors and indoors without any study of indoor/outdoor building-shell penetration relationships, their contribution to total PM, transport properties of BioPM, possible ambient BioPM sources, or optimal ambient BioPM control.

Mold and pollen particles that are known to exist in a size range greater than 10 μm are believed to be present in the fine PM size range, and make up part of the total BioPM. This hypothesis was tested along with the broad project goals that focus attention to BioPM as a potential source of exposure of airborne biocontaminants. The goals for this research include development of testing methods and procedures to provide standardized tools and protocols for biocontaminant research and evaluation and studies of the relationship between environmental conditions, substrate properties, and biocontaminant growth and death. The data generated by this cooperative research are to be used as a basis for guidance, criteria, and the development of effective engineering control methods for prevention and mitigation of indoor air biocontamination. The research objectives include the development of a set of analytical methods and the comparison of impactor media and sample size, and the quantification of the relationship between outdoor and indoor levels of bioaerosols.

MATERIALS AND METHODS

Indoor and outdoor particle samples were collected using an Andersen eight-stage nonviable cascade impactor. Each impactor stage varied in the size of particle that it captured. The size fractions ranged from 0.4–0.7 μm for the smallest to 5.8–9.0 μm for the largest and are listed in Tables 1–3.

The Andersen cascade impactor collected nonviable BioPM samples from a house indoor air environment and ambient outdoor air from directly outside of the same

Table 1. Indoor/outdoor mass and assay results with silicon spray on foil impactor media.

Indoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	3.6	0.012	0.02	2.9	2.1	0.03	47
1	5.8–9.0	7.4	2.7	0.012	0.02	7.6	4.1	1.8	51
2	4.7–5.79	5.25	0.8	0.011	0.02	5.7	0.5	1.3	22
3	3.3–4.69	4	1.8	0.016	0.09	7.4	0.0	0.4	53
4	2.1–3.29	2.7	0.0	0.017	0.11	1.4	0.0	2.3	14
5	1.1–2.09	1.6	0.9	0.003	0.01	5.8	2.3	0.2	BDL
6	0.7–1.09	0.9	1.9	BDL	0.01	8.7	0.7	0.1	15
7	0.4–0.69	0.55	3.3	0.000	0.001	3.4	0.6	0.8	BDL
Backup	0–0.39	0.2	4.8	0.001	0.002	18.0	3.1	0.4	28
Outdoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	1.2	0.003	0.01	1.3	0.0	1.2	BDL
1	5.8–9.0	7.4	1.2	0.000	0.01	6.7	2.1	2.2	29
2	4.7–5.79	5.25	0.8	0.001	0.14	2.5	0.0	2.8	24
3	3.3–4.69	4	1.6	0.001	0.02	0.3	0.0	0.3	34
4	2.1–3.29	2.7	1.7	0.002	0.02	0.8	0.2	1.7	7
5	1.1–2.09	1.6	2.2	0.001	0.01	2.1	0.6	1.2	17
6	0.7–1.09	0.9	2.9	0.000	0.0003	1.4	0.0	0.7	BDL
7	0.4–0.69	0.55	7.1	BDL	0.002	6.1	5.1	0.0	21
Backup	0–0.39	0.2	7.5	BDL	0.001	12.0	8.5	0.0	BDL

Note: BDL is beyond detectable limits.

house. Samples were collected inside and outside of a residence simultaneously using the eight-stage cascade impactor and a flow rate of 0.47 L/sec. Silicon-coated aluminum foil disks were used in the sample collection of Tables 1 and 2, and a glass fiber filter was inserted at the final backup stage to ensure complete capture at this smallest size fraction and to comply with the impactor manufacturer's specifications (Andersen Instrumentation). Glass fiber filters were seated on all impactor stages of the air samples listed in Table 3 as a comparison of impaction media efficiency.

All impactor media were equilibrated for 24 hr in a temperature (25 ºC)- and humidity (35%)-controlled room and then weighed prior to sampling. To increase measurement accuracy, samples were collected from 62 to 110 hr to offset variation in bioaerosol concentrations, which can vary substantially from hour to hour.[7] After collection, the samples were allowed to equilibrate for 24 hr, weighed, and their total mass load was determined. The entire filter or collection media was suspended in sterile, pyrogen-free, glucan-free water and analyzed for ragweed antigen, beta-1-3 glucans, fungal (*Alternaria alternata* and *A. niger*) antigens, lipopolysaccharide endotoxin, and total protein.

The antigens were analyzed using a modification of the Food and Drug Administration Center for Biological Evaluation and Research (CBER)[8] procedure for the enzyme-linked immunosorbent assay inhibition. This polyclonal assay for multiple antigens detection was selected over the monoclonal assay (detects one specific antigen) to include additional antigens, and it results in a lower minimum detection limit. The samples were analyzed first by coating a 96-well microtiter plate for 24 hr with the reference antigen. The plate wells were washed with phosphate-buffered saline (PBS) with 0.05% Tween 20 solution (PBS-Tween). The test sample dilutions and reference standard dilutions were added to the plate along with the standard antibody and incubated for 4–6 hr. The plate wells were washed with a PBS-Tween solution, the conjugate solution (goat, anti-rabbit IgG-alkaline phosphatase) was added, and the sample was incubated overnight. The plate wells were washed with a PBS-Tween solution, and the substrate (*p*-nitrophenyl phosphate) was added. The level of color change was quantified using a Bio-Tek Microplate reader, measuring the optical density at 405 nm at

Table 2. Indoor/outdoor mass and assay results with silicon spray on foil impactor media.

Indoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	1.3	0.005	0.02	2.5	1.3	ND	8
1	5.8–9.0	7.4	NA	0.001	0.02	6.3	0.7	ND	8
2	4.7–5.79	5.25	0.7	0.001	0.02	4.8	1.6	ND	16
3	3.3–4.69	4	NA	0.002	0.06	6.4	1.6	ND	4
4	2.1–3.29	2.7	0.9	0.001	0.05	1.2	2.2	ND	13
5	1.1–2.09	1.6	1.0	0.001	0.01	3.8	0.01	ND	9
6	0.7–1.09	0.9	1.0	0.000	0.003	3.3	0.6	ND	11
7	0.4–0.69	0.55	1.8	0.000	0.001	0.9	1.7	ND	7
Backup	0–0.39	0.2	NA	0.001	0.003	1.6	4.2	ND	BDL
Outdoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	1.2	0.005	0.02	5.6	0.6	ND	BDL
1	5.8–9.0	7.4	0.6	0.004	0.02	5.2	0.3	ND	ND
2	4.7–5.79	5.25	1.0	0.002	0.09	31.5	3.6	ND	8
3	3.3–4.69	4	1.1	0.005	0.15	55.7	2.3	ND	7
4	2.1–3.29	2.7	1.1	0.004	0.07	84.8	0.8	ND	3
5	1.1–2.09	1.6	0.8	0.001	0.01	67.4	5.2	ND	6
6	0.7–1.09	0.9	1.3	0.000	0.004	61.3	1.9	ND	BDL
7	0.4–0.69	0.55	2.8	0.000	0.002	0.7	1.2	ND	BDL
Backup	0–0.39	0.2	NA	0.000	0.003	10.7	3.6	ND	ND

Note: NA is not available; ND is no data; BDL is beyond detectable limits.

different time intervals over the following hour. The assay determines the relative potency of the antigen in the test sample compared with a standard antigen preparation. The standard antigen and antibody were purchased from Greer Laboratories.

Endotoxins were measured using the *Limulus* Amebocyte Lysate (BioWhittaker). The level of endotoxin in a sample was determined by the reaction of endotoxin in the specimen with lysate and a substrate, producing a color change over time, compared with similar reactions of a known standard endotoxin reference. The level of color change was quantified using a Bio-Tek Microplate reader measuring the optical density at 405 nm.

Protein was measured using the Nano-Orange Protein Quantification Kit (Molecular Probes). Quantification of the level of protein in a sample was obtained by the reaction of protein in the specimen with a diluted Nano-Orange agent heated to 950 °C for 10 min and then allowed to cool to room temperature. A fluorescent reaction was produced and compared with a known standard of bovine serum albumin. The level of fluorescence was measured using a Turner digital fluorometer model 450. Protein is used as a general indicator of biologically based PM.

RESULTS

The results of three series of indoor and outdoor measurements are listed in Tables 1–3. Samples were collected inside and outside of a residence simultaneously using the eight-stage cascade impactor and a flow rate of 0.47 L/sec. For each table, data are listed by column, identified by the abbreviation of each analytical procedure or method of sample collection. The abbreviations signify the following information: (1) Stage #, for the Andersen cascade impactor collection stage; (2) Mid-Pt, for the midpoint of the size range of the aerodynamic particle sizer; (3) Mass, for the total particulate mass; (4) LPS, for lipopolysaccharide of endotoxin; (5) Glucan, for beta-1-3 glucans; (6) Ragweed Ag, for ragweed antigen; (7) Alt Ag, for *Alternaria alternata* antigen; (8) An Ag, for *Aspergillus niger* antigen; and (9) Protein, for total protein.

The first line of data in each table lists information for Stage #0, which captures and eliminates all particles larger than 9 μm. Analysis of the impacted mass of particles taken from this stage was included as an additional or 9th stage and source of information. The other eight stages collected particles in the following μm size ranges: (stage 1) 5.8–9.0, (stage 2) 4.7–5.79, (stage 3) 3.3–4.69,

Table 3. Indoor/outdoor mass and assay results with glass fiber impactor media.

Indoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	106.2	0.0020	0.02	0.02	0.9	0.4	449
1	5.8–9.0	7.4	39.5	0.0026	0.01	0.3	0.9	0.1	68
2	4.7–5.79	5.25	12.8	0.0022	0.02	0.0	1.6	0.0	60
3	3.3–4.69	4	5.8	0.0062	0.04	0.0	0.5	0.0	ND
4	2.1–3.29	2.7	88.5	0.0033	0.02	0.1	0.3	0.1	333
5	1.1–2.09	1.6	2.3	BDL	0.01	0.6	0.1	0.9	ND
6	0.7–1.09	0.9	25.4	0.0018	0.00	0.1	0.5	0.2	ND
7	0.4–0.69	0.55	55.4	0.0005	0.00	0.0	3.2	0.1	166
Backup	0–0.39	0.2	NA	0.0011	0.00	1.2	1.2	1.7	2
Outdoor									
Stage #	**Size Range (μm)**	**Mid-Pt (μm)**	**Mass (μg/m³)**	**LPS (ng/m³)**	**Glucan (ng/m³)**	**Ragweed Ag (ng/m³)**	**Alt Ag (ng/m³)**	**An Ag (ng/m³)**	**Protein (ng/m³)**
0	>9.0	9	30.1	0.007	0.17	0.6	0.1	0.1	177
1	5.8–9.0	7.4	59.3	0.007	0.05	1.1	0.1	0.6	21
2	4.7–5.79	5.25	1.0	0.007	0.09	1.5	0.7	0.2	18
3	3.3–4.69	4	1.7	0.008	0.11	2.0	0.1	0.0	17
4	2.1–3.29	2.7	1.6	0.004	0.08	0.0	0.0	0.3	21
5	1.1–2.09	1.6	2.7	0.000	0.00	2.6	0.04	0.2	ND
6	0.7–1.09	0.9	17.3	0.001	0.01	0.7	0.2	0.6	28
7	0.4–0.69	0.55	3.5	0.001	0.00	0.0	0.0	1.4	19
Backup	0–0.39	0.2	NA	0.002	0.01	0.03	1.6	0.0	29

Note: NA is not available; ND is no data; BDL is beyond detectable limits.

(stage 4) 2.1–3.29, (stage 5) 1.1–2.09, (stage 6) 0.7–1.09, (stage 7) 0.4–0.69, and backup (stage 8) 0.1–0.39. It should be understood that these size intervals are approximations and, although specified by the manufacturer, the midpoint (which is also specified by the manufacturer, Andersen Instrumentation) is a better indicator of the average size of particle that is separated from the sample airstream.

The samples listed in Table 1 were collected from October 29 to November 1, 1999, over a 62-hr sampling period. The total sample volume of 156,240 L passed through the impactor, depositing the sample BioPM onto silicon-coated aluminum foil disks. The samples listed in Table 2 were collected from November 3 to November 8, 1999, over a 110-hr sampling period. The total sample volume of 277,200 L passed through the impactor, depositing the BioPM onto silicon-coated aluminum foil disks. The length of sample collection and total sample volume was increased for the measurements listed in Table 2. Although the sample volume for Table 2 was greater than that for Table 1, the recorded mass results of Table 2 were comparable to Table 1, and the assay results for *A. niger* antigen were consistently lower.

The samples listed in Table 3 were collected from November 11 to November 15, 1999, over a 92-hr sampling period. The total sample volume of 231,840 L passed through the impactor, depositing the BioPM on glass fiber disks at all eight stages. The results for this sample indicated higher amounts of mass, while other measured BioPM components were relatively consistent with the results listed in the previous two tables.

The data in Table 1 were analyzed by calculating indoor-to-outdoor concentrations for each size fraction. From data recorded in Table 1, the ratio of indoor concentrations divided by outdoor concentrations is shown in Figure 1, where BioPM concentrations that are greater indoors have a ratio greater than 1. This analysis of indoor-to-outdoor ratios may indicate the presence of indoor BioPM in excess of outdoor levels in a number of the categories assayed. Data taken from Table 1 were also used for Figures 2 and 3, which depict the contribution of each BioPM category assayed by size fraction. The BioPM constituents profiled in Figures 2 and 3 indicate significant differences between indoor and outdoor concentrations. The data imply that the constituents of the indoor sample of BioPM are significantly different from those of the outdoor sample.

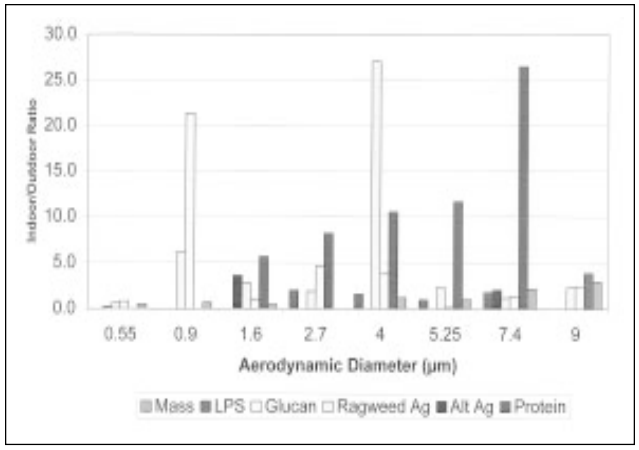

Figure 1. Indoor/outdoor ratio.

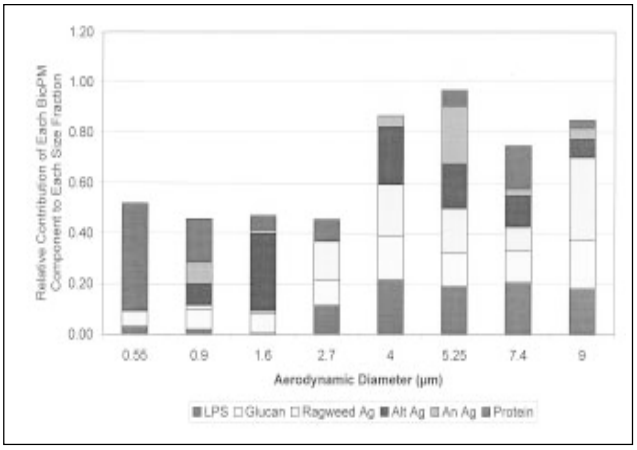

Figure 3. Biocontaminant contribution by size fraction (outdoor).

DISCUSSION

The test results listed above were from a preliminary effort to quantify BioPM by size fraction and mass and available assays of constituent pollen, mold, and endotoxin. Data for variations in results by sample volume, impactor media, and component assay were compared for the purpose of identifying an optimal analytical method. After making these comparisons, it became evident that, although the glass fiber media indicated an advantage in capturing a greater mass of PM, no advantage was indicated in any of the assay data from stages 1–7. But to accurately compare this impactor media, additional research is necessary. At this time, it is not clear why the presence of a larger sample mass did not translate into a larger proportion of pollen, mold, and endotoxin. It may have to do with the nature of the glass fiber filter, sample preparation, fiber uptake/release of PM, variation in extraction efficiency, or the concentrations of BioPM. Seasonal flux of airborne pollen and nonsimultaneous sampling periods for the three series of samples make the comparison of ragweed pollen difficult.

Comparison of indoor-to-outdoor samples within each series of measurements did not indicate a general trend. In some instances, outdoor samples were greater and in other instances, indoor samples were greater within the same category of assayed results. The presence of mold, pollen, and endotoxin within all size ranges tested from the midpoint of 0.2 to 7.4 µm was demonstrated repeatedly. These respirable allergens listed in the results shown in Tables 1–3 were found in both indoor and outdoor BioPM. *A. alternata*, normally found to be ellipsoidal in shape from 18 to 83 µm × 7 to 18 µm, have been shown in Tables 1–3 to be present in size fractions below 5.25 µm, suggesting that these highly allergenic spores are broken into fragments in the respirable size range.[9,10] Analogous findings are listed in the previous sections for ragweed pollen, and although seasonal variations of bioaerosols would affect the concentrations and constituent ratios of both ambient and indoor BioPM, documenting the effects of seasonal variation was beyond the purpose of this study.

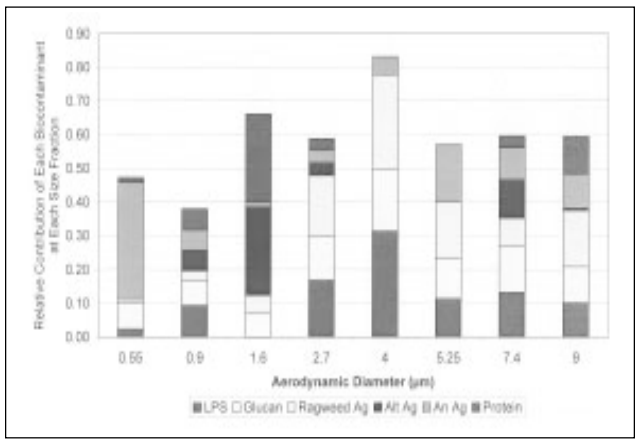

Figure 2. Biocontaminant contribution by size fraction (indoor).

The comparison of indoor and outdoor levels of bioaerosols is often used as an indicator of possible indoor contamination. The comparison made in Figure 1 of indoor/outdoor component ratios of BioPM indicated numerous instances in which outdoor levels were greater (the ratio was less than 1), as well as instances in which indoor levels were greater (the ratio was greater than 1). The relationship between indoor and outdoor BioPM could not be determined from these preliminary test results. Although samples were collected simultaneously, variation in BioPM could have accounted for differences in sample results. However, with further analytical refinement and optimization of sample collection and analysis, a better judgment of these relationships can be accomplished.

CONCLUSIONS

The results indicate that sizable fractions of the fine PM sampled in both indoor and outdoor samples were of biological origin. These results are preliminary and, although

they establish the presence of a biological component with indoor and outdoor fine PM, additional research is needed to (1) further develop the measurement method, (2) quantify the relationship between indoor and outdoor levels of the BioPM, and (3) determine the fraction of ambient and indoor PM that is biological. The importance of answering these questions is evidenced by escalating occurrences of asthma, the potential for exposure in the indoor environment, and the ability of BioPM to cause allergic, toxic, and infectious responses in exposed individuals.

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